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VISTA expressed in tumor cells regulates T cell function(Abstract_要旨)

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論文題目	VISTA expressed in tumor cells regulates T cell function 腫瘍細胞に発現する免疫補助シグナル分子 VISTA（B7-H5）の機能及び発現メカニズムの解明		

（論文内容の要旨）

Tumor-induced immune suppression is a major obstacle for cancer immunotherapies that seek to eliminate cancer cells. Various mechanisms contribute to this immune activity. New immunotherapies targeting immune checkpoints, including members of the B7 and CD28 families such as PD-1 and PD-L1, have been exploited with therapeutic benefit in human cancers. VISTA is a newly discovered B7 family member (B7-H5) whose function has been primarily explored in immune cells. However, its expression on tumor cells and the associated regulatory mechanisms remain unclear.

Ovarian cancer is the most lethal gynecologic cancer worldwide and the fourth most common cause of cancer-related death in women. Although chemotherapy is effective in the majority of the ovarian cancer patients, more than 70% of patients suffer from recurrence and eventually develop chemoresistance. Endometrial cancer is another of the most common gynecological cancers. The prognosis of low-risk endometrial cancer is generally favorable. However, for high-risk endometrial cancer patients, chemotherapeutic options are limited, and no molecular therapies have been approved. Therefore, the development of novel therapeutic strategies, including immunotherapy, is urgently required.

In this paper, the expression of VISTA was firstly detected in the ovarian and endometrial cancer patient samples using immunohistochemical staining. The VISTA IHC score was significantly higher in cancer samples than in normal tissues. Gene expression of VISTA in the GSE microarray dataset (GSE17025) was consistent with the IHC data.

Since VISTA was detected on most of the ovarian and endometrial cancer cell lines, sh-VISTA cell lines were established to assess the functional effect of VISTA expression. As a result, silencing of VISTA expression in tumor cells by sh-RNA system increased T cell proliferation and cytokine production.

For understanding the mechanism of VISTA expression, cytokine inducement and miRNA regulation were firstly investigated, but no significant effect was observed. On the other hand, decitabine-treated endometrial cancer cell lines showed increased VISTA expression. Following this clue, the methylation status of the VISTA promotor was focused on.

Among three selected specific promotor regions, one of them showed significantly different methylation status between VISTA-high and VISTA-low cancer cell lines. This result was further confirmed in the endometrial cancer patients, suggesting that VISTA expression in endometrial cancer is controlled by DNA methylation of its promoter.

VISTA overexpressing mouse ovarian cancer cell lines were used to study the function of VISTA in tumor cells in vitro, T cell cytotoxicity and proliferation assays were performed. Higher levels of target cell lysis were observed in cultures with control tumor cells than in those with VISTA overexpressing tumor cells, indicating that VISTA in tumor cells inhibits antigen-specific cytolysis of CD8+ T cells, furthermore, a suppressive status of T cell proliferation was observed in co-cultured with VISTA overexpressed tumor cells.

The effect of VISTA expression in tumor cells in vivo was studied using VISTA overexpressed mouse ovarian cancer cell line-induced peritoneal disseminated tumor models. VISTA overexpression significantly decreased the number of CD8+ T cells at the tumor site, while there was no significant difference in the number of CD4+ T cells. Moreover, the percentage of IFN-γ-producing CD8+ T cells at the tumor site was reduced when they were inoculated with VISTA overexpressing tumor cells. Furthermore, no survival difference was detected in immunodeficient mice when inoculated with VISTA overexpressed cell line or control cell line, but in immunocompetent mice, survival of mice inoculated with VISTA overexpressing cells was significantly shortened. These results suggested that VISTA expression in the tumor induces immunosuppression in host immune cells, leading to a poor prognosis. Collectively, these data indicated that VISTA in tumor cells negatively regulates antitumor immunity in ovarian cancer.

The effect of VISTA expression in tumor cells in vivo was studied using VISTA overexpressed mouse ovarian cancer cell line-induced peritoneal disseminated tumor models. VISTA overexpression significantly decreased the number of CD8+ T cells at the tumor site, while there was no significant difference in the number of CD4+ T cells. Moreover, the percentage of IFN-γ-producing CD8+ T cells at the tumor site was reduced when they were inoculated with VISTA overexpressing tumor cells. Furthermore, no survival difference was detected in immunodeficient mice when inoculated with VISTA overexpressed cell line or control cell line, but in immunocompetent mice, survival of mice inoculated with VISTA overexpressing cells was significantly shortened. These results suggested that VISTA expression in the tumor induces immunosuppression in host immune cells, leading to a poor prognosis. Collectively, these data indicated that VISTA in tumor cells negatively regulates antitumor immunity in ovarian cancer.

Finally, given the ability of VISTA to inhibit T cell responses, the effect of blocking VISTA was investigated. Mice inoculated with VISTA overexpressed ovarian cancer cell line treated with anti-VISTA antibody exhibited significantly prolonged survival in comparison with the control group.

In conclusion, the VISTA-mediated immune inhibitory pathway in tumor cells regulates antitumor immunity, and blocking of VISTA in tumor cells may provide a promising immunotherapeutic strategy for improving the antitumor response.

（論文審査の結果の要旨）

本論文では、腫瘍細胞が免疫抑制性補助シグナル分子 VISTA（B7-H5）を発現していることを示し、さらに同発現が宿主免疫にどのように関与しているのかを明らかにすることを目的として研究を行った。

その結果 VISTA は、卵巣がんや子宮体がんに高発現していることを見つけ、さらに VISTA の発現は、同プロモータ領域のメチル化状態に関連することを示した。また VISTA を強制発現した腫瘍細胞株は、親株と比して、T 細胞との共培養実験にて有意に T 細胞の増殖や機能を抑制した。さらに同細胞株を用いたマウス担癌モデルでは、親株に比して、腫瘍内の CD8 陽性 T 細胞の浸潤を抑えるとともに、生存期間も短縮したが、抗 VISTA 抗体を投与することによって生存期間が延長した。

これらの結果から、従来、免疫細胞に発現することが知られていた VISTA が、エピジェネティックに腫瘍細胞にも発現すること、さらにその発現は T 細胞免疫を抑制し腫瘍増殖に関与することから、新たながん治療の標的として有望である可能性が示された。

以上の研究は腫瘍細胞に発現する免疫補助シグナル分子 VISTA 発現の解明に貢献し腫瘍免疫研究の発展に寄与するところが多い。

したがって、本論文は博士（医学）の学位論文として価値あるものと認める。

なお、本学位授与申請者は、平成３０年１２月２５日実施の論文内容とそれに関連した試問を受け、合格と認められたものである。

要旨公開可能日：　　年　　月　　日以降